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(54) Title: PHOTOACTIVATABLE COMPOUNDS FOR THE PREVENTION OF INTIMAL HYPERPLASIA AND OTHER DISEASES

(57) Abstract

A broad class of photosensitive compounds having enhanced *in vivo* target tissue selectivity and versatility in photodynamic therapy. Many furocoumarin compounds, such as psoralens, exhibit cytostatic activity when photoactivated but exhibit little *in vivo* specificity for selectively accumulating in any particular target tissue such as atheromatous plaques. Reactive Oxygen Producing Photosensitizers ("ROPPs") are photoactivatable compounds having an affinity for hyperproliferating cells (such as atheromatous plaque cells), which when photoactivated, produce cytotoxic reaction products. The photoactivity of a ROPP, such as a porphyrin, may be reduced by metalating the porphyrin while the selective affinity of the metalized ROPP for hyperproliferating tissue remains substantially unchanged. By linking a furocoumarin compound to a ROPP to form a F-ROPP, the cytostatic properties of the furocoumarin portion of the F-ROPP can be exploited while the selective affinity of the ROPP portion of the compound for hyperproliferating cells such as atheromatous plaque provides enhanced tissue selectivity without cytotoxicity. *In vivo*, certain F-ROPPs may be forced to selectively accumulate in a target tissue by illuminating only the target tissue with light having a wavelength operable for photoactivating the F portion of the F-ROPP thereby causing the F-ROPP to either form a monoadduct with or cross-link the cellular DNA in the target tissue. Light of a second wavelength can then be delivered to the target tissue to photoactivate the ROPP portion causing further interference with cellular activity.

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PHOTOACTIVATABLE COMPOUNDS FOR THE PREVENTION OF INTIMAL HYPERPLASIA AND OTHER DISEASES

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to photoactivatable compounds and to methods for using the compounds for diagnosing and treating medical conditions.

2. Prior Art

Photodynamic Therapy (PDT) is used for treating various diseases including cancer, psoriasis, vascular disease, non-cancerous hyperplastic disease such as benign prostatic hyperplasia, macular degeneration, glaucoma, and certain viral infections. PDT requires concentrating a photosensitizer drug in a target tissue then photoactivating the compound with a device which includes a light source providing light at a particular wavelength and power level. The drugs administered for PDT are commonly known as photosensitizers (PS) due to their inherent ability to absorb photons of light and transfer that energy to oxygen which then converts to a cytotoxic or cytostatic species. Table 1 presents a list of classes of photosensitizer compounds commonly employed in PDT, which PS's are referred to hereinafter in the alternative as "ROPPs" (Reactive Oxygen Producing Photosensitizer molecules) and "LEPs" (Light Emitting Photosensitive molecules). While not exhaustive, the list of PDT photosensitizer drugs presented in Table 1 is exemplary of the variety of ROPPs and LEPs currently used in the art.

The photoactivating device employed for PDT usually comprises a monochromatic light source such as a laser, the light output of which may be coupled

to an invasive light delivery catheter for conduction and delivery to a remote target tissue. Such interventional light delivery catheters are well known in the art and are described, for example, in U.S. Patents 5,169,395; 5,196,005; and 5,231,684. Other devices which are frequently used in conjunction with a light source and light delivery catheter include drug delivery devices and/or a balloon perfusion catheter (U.S. Patent 5,213,576) and/or various medicament-dispensing stents for the slow localized release of the photosensitizer. PDT is presently an approved procedure in Canada, Japan, and The Netherlands for the treatment of various cancers.

In addition to cancer therapy, PDT is being tested for the treatment of psoriasis. Extra-corporal PDT of blood is being evaluated for the prevention of intimal hyperplasia following transplant surgery. PDT is also being evaluated for the treatment of vascular disease; most commonly the prevention of intimal hyperplasia following angioplasty. ROPPs are presently in clinical trials for the treatment of cutaneous cancers such as basal cell carcinoma, basal cell nevus syndrome, squamous cell carcinoma, and AIDS related Kaposi's sarcoma. ROPPs are also being investigated for the treatment of a cancer precursor, Barrett's esophagus. In addition, ROPPs may have utility for treating invasive cancers, cancer precursors, psoriasis, non-cancerous urological disorders, viral inactivation, macular degeneration, glaucoma and various vascular diseases.

1 **Table 1: ROPPs and LEPs**

Pyrrole-derived macrocyclic compounds Naturally occurring or synthetic porphyrins and derivatives thereof (1)* Naturally occurring or synthetic chlorins and derivatives thereof (2) Naturally occurring or synthetic bacterio-chlorins and derivatives thereof (3) Synthetic isobacteriochlorins and derivatives thereof (4) Phthalocyanines and derivatives thereof (5) Naphthalocyanines and derivatives thereof (6) Porphycenes and derivatives thereof (7) Porphycyanines and derivatives thereof (8) Pentaphyrin and derivatives thereof (9) Sapphyrins and derivatives thereof (10)	Texaphyrins and derivatives thereof (11) Phenoxazine dyes and derivatives thereof (12) Phenothiazines and derivatives thereof (13) Chalcoorganapyrylium dyes and derivatives thereof (14) Triarylmethanes and derivatives thereof (15) Rhodamines and derivatives thereof (16) Fluorescenes and derivatives thereof (17) Azaporphyrins and derivatives thereof (18) Benzochlorins and derivatives thereof (19) Purpurins and derivatives thereof (20) Chlorophylls and derivatives thereof (21) Verdins and derivatives thereof (22)

2

3

4

5 * (m) refers to the compound having molecular structure indicated at (m) in the

6 specification where m is an integer between 1 and 22.

ROPPs and LEPs such as those indicated in Table 1, and as illustrated in Figures 1-23, have been shown to selectively accumulate, both *in vitro* and *in vivo*, in catheter induced atheromatous plaques in rabbit and swine models as evidenced by laser induced fluorescence and chemical extraction (HL Narciso, et al, Retention of tin ethyl etiopurpurin (SnET2) by atheromatous plaques: Studies in vitro & in vivo rabbits, *Proceedings of SPIE: Diagnostic and Therapeutic Cardiovascular Interventions IV*, 1994, 2130:30-41). *In vitro* studies utilizing human cadaver aortas demonstrate the passive accumulation of photosensitizers such as ROPPs and LEPs into naturally occurring atheromatous plaques. Certain ROPPs and LEPs have the ability to penetrate the nuclear membrane within a cell and to intercalate into the nuclear DNA, particularly ROPPs bearing a positive charge (cationic).

Psoralen-type compounds have also been investigated for their ability to prevent intimal hyperplasia. Psoralens and other furocoumarins (furane fused to coumarin and derivatives thereof) are also photosensitive compounds which have been used in the treatment of psoriasis for over 40 years. Such psoralen-based phototherapy is alternatively referred to herein as PUVA; Psoralen activated with UltraViolet A light. An exemplary list of some furocourmarin compounds is presented in Table 2. Systemically administered psoralen-type compounds penetrate the nuclear membrane of cells and may intercalate with the nuclear DNA in target tissue cells. Following intercalation with the target tissue's nuclear DNA, the psoralen compound is photoactivated with ultraviolet light or short wavelength visible light (see, for example, FP Gasparro, et al, The excitation of 8-Methoxypsoralen with visible light: Reversed phase HPLC quantitation of monoadducts and cross-links, *Photochem. Photobiol.*, 1993, 57(6):1007-1010.), which UV light is preferably delivered only to the target

tissue by a light delivery catheter or similar delivery device, to cause DNA crosslinking and ultimately a mutagenic effect in the cells comprising the target tissue. (KL March, et al, 8-Methoxypsoralen and longwave ultraviolet irradiation are a novel antiproliferative combination for vascular smooth muscle, *Circulation*, 1993, 87:184-91; BE Sumpio, et al, Control of smooth muscle cell proliferation by psoralen photochemotherapy, *J. Vasc. Surg*, 1993, 17:1010-1018; KW Gregory, et al, Photochemotherapy of intimal hyperplasia using psoralen activated by ultraviolet light in a porcine model, *Lasers in Surg. Med.*, 1994, (Suppl 6):12 Abstract).

Furocoumarins are photochemical agents showing potential for both diagnostic and therapeutic applications in medicine. The DNA cross-linking by furocoumarins such as described above proceeds by a two step process. Following injection of the furocoumarin into the body of an animal, the (planar) furocoumarin molecule first intercalates within the double helix of intracellular DNA or RNA. Following intercalation, the covalent addition of the furocoumarin to the polynucleic acid is achieved through the addition of light energy within the absorption band of the specific furocoumarin. Either furocoumarin -RNA or -DNA monoadducts or cross-links may be created upon illumination of the intercalated species. By forming covalent cross-links with base-pair structures, furocoumarins can alter the metabolic activity of a cell and induce cytostasis (GD Cimino, HB Gamper, ST Isaacs, JE Hearst, Psoralens as photoactive probes of nucleic acid structures and function: Organic chemistry, and biochemistry, *Ann. Rev. Biochem.*, 1985, 54:1154-93).

Table 2: Furocoumarins‡

Compounds containing Furocoumarin sub-components (23)*
Psoralens and derivatives thereof (24)
Isopsoralens (angelicins) and derivatives thereof (25)
Pseudopsoralens and derivatives thereof (26)
Pseudoisopsoralens and derivatives thereof (27)
Allopsoralens and derivatives thereof (28)
Pseudoallopsoralens and derivatives thereof (29)

* (m) refers to the compound having the structure indicated at Figure m in the appended figures where m is an integer $23 \leq m \leq 29$.

‡ The furocoumarins may be either naturally occurring or synthetic.

Coronary artery disease is thought to be initiated by a disruption of fatty streaks which form early in life on the vessel wall which disruption, in turn, promotes thrombus formation. Over time the thrombus becomes organized and provides structure for the accumulation of fatty lipids, foam cells, cholesterol, calcium, fibrin, and collagen. A fibrous cap forms over this collection of lipid-rich material. Periodically this fibrous cap ruptures; releasing some of the lipid-rich material and exposing the remaining plaque materials to the circulating blood. Growth factors within the blood initiate the migration of smooth muscle cells (SMCs), from the media to the intima where proliferation of the SMCs begins. The ulcerated plaque induces the deposition of platelets and thrombus formation in a "response to injury" mode. This cycle recurs until eventually the plaque ruptures, the distal coronary artery is occluded by an thrombus and a heart attack occurs (V. Fuster, et al, Clinical-Pathological Correlations of Coronary Disease Progression and Regression, *Supplement to Circulation*, Vol. 86, No. 6, 1992:III-1-III-11 and JJ Badimon, Coronary Atherosclerosis, A Multifactorial Disease, *Supplement to Circulation*, Vol. 87, No. 3, 1993:II-3-II-16).

Restenosis occurs when coronary disease is treated with an interventional therapy such as Percutaneous Transluminal Coronary Angioplasty, PTCA, or atherectomy, or laser angioplasty, or stenting, or a myriad of newer technologies. Restenosis refers to the over- aggressive autogenous repair of an injury to a blood vessel by the body. Intimal hyperplasia or the hyperproliferation of medial (and possibly adventitial) smooth muscle cells (SMCs,) is a major contributing factor to restenosis. Hyperproliferating SMCs form a neo-intima which can reduce the bore of the arterial lumen and thus the capacity of the artery to deliver oxygen rich blood. This

reduction in cross-sectional luminal area can be more severe than the original constricted area which was treated. The foregoing problems are representative of some medical conditions which the compounds of the present invention may have particular application.

DNA cross-linking by furocoumarins results in the reduction of smooth muscle cell (SMC) proliferation and, since their DNA cross-linking activity is cytostatic, furocoumarins may have certain advantages over cytotoxic photosensitizers (ROPPs and LEPs) in the prevention of intimal hyperplasia as described by March, et al, U.S. Patent 5,116,864 and Deckelbaum, et al, U.S. Patent 5,354,774 the teachings of which patents are incorporated herein by reference thereto. The cytotoxicity of ROPPs and LEPs currently used in PDT results in the extravasation of intracellular organelles, cytoplasm, and cytokines which, in turn, elicits an inflammatory response. The inflammatory response elicited by extravasation of cellular contents is hypothesized as a key contributing factor to restenosis. The disadvantage of employing psoralens to prevent restenosis (when compared to photosensitizers such as ROPPs and LEPs) is that psoralens do not exhibit a selective affinity for atheromatous plaques over normal intimal tissue.

SUMMARY OF THE INVENTION

It is a primary object of the present invention to provide a photoactivatable compound which can be used to treat a variety of diseases.

It is an object of the present invention to provide a photoactivatable therapeutic compound which causes cytostasis but not cytolysis when bound to a cell and activated with light.

It is another object of the present invention to provide a photoactivatable compound which has a selective affinity for rapidly proliferating cells.

It is still a further object of the present invention to provide a photoactivatable compound which will reduce the incidence of restenosis following phototherapy of atheromatous plaque.

It is a further object of the present invention to provide a photoactivatable compound which can cause cytostasis when activated by a specific wavelength of light.

It is still a further object of the present invention to provide a photoactivatable compound which can cause cytostasis when activated by one particular wavelength of light and cause cytolysis when activated with light having a different wavelength.

It is yet a further object of the present invention to provide a method for treating such diseases as atherosclerosis, restenosis, cancer, cancer precursors, noncancerous hyperproliferative diseases, psoriasis, macular degeneration, glaucoma and viruses employing photoactivatable compounds.

It is a further object of the present invention to provide a method for employing such photoactivatable compounds for diagnosing such diseases as atherosclerosis, restenosis, cancer, cancer precursors, noncancerous hyperproliferative diseases, psoriasis, macular degeneration, glaucoma and viruses.

The features of the invention believed to be novel are set forth with particularity in the appended claims. However, the invention itself, both as to composition and manner of use, together with further advantages of these compounds may best be understood by reference to the following description of preferred embodiments.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1-22 present the chemical structures of various photosensitive pyrrole-derived macrocyclic compounds which exhibit as follows:

Figure 1 illustrates the chemical structure of photoactivatable compositions comprising a porphyrin core.

Figure 2 shows clorin compounds.

Figure 3 shows bacterioclorin-derived compounds.

Figure 4 illustrates isobacteriochlorin compounds

Figure 5 shows phthalocyanines.

Figure 6 shows naphthalocyanine compounds.

Figure 7 illustrates porphycene-containing compounds.

Figure 8 is porphycyanine compounds.

Figure 9 is pentaphyrin derivatives.

Figure 10 shows sapphryin and derivatives thereof.

Figure 11 illustrates texaphyrin and derivatives thereof.

Figure 12 shows the chemical structures of phenoxazine dyes and derivatives thereof.

Figure 13 is phenothiazine and derivatives thereof.

Figure 14 illustrates chalcoorganapyrylium dyes.

Figure 15 shows triarylmethane derivatives.

Figure 16 gives the structure of rhodamine and derivatives thereof.

Figure 17 is fluorescene derivatives.

Figure 18 shows azaporphyrin and derivatives thereof.

Figure 19 shows benzochlorin and derivatives thereof.

Figure 20 illustrates the structure of purpurin and derivatives thereof.

Figure 21 shows chlorophyll and derivatives thereof.

Figure 22 is verdin and derivatives thereof.

Figure 23 shows the chemical structure of compounds containing furocoumarin sub-components.

Figure 24 illustrates psoralens and derivatives thereof.

Figure 25 shows the structure of isopsoralens (angelicins) and derivatives thereof.

Figure 26 is the chemical structure of pseudopsoralens and derivatives thereof.

Figure 27 illustrates the chemical structure of pseudoisopsoralen compounds.

Figure 28 shows allopsoralen and derivatives thereof.

Figure 29 is pseudoallopsoralen and derivatives thereof.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

A problem encountered when using conventional cytotoxic photosensitizer compounds such as those listed in Table 1 for PDT is the post-administration inflammatory sequella such as restenosis of a blood vessel. While photosensitizers such as ROPPs and LEPs exhibit enhanced selectivity and avidity for rapidly proliferating cells in comparison with normal, more quiescent cells, the cytotoxic and cytolytic activity of such compounds may be undesirable.

A problem encountered when using PUVA for the treatment of hyperproliferative conditions is that furocoumarins exhibit little, if any, specificity and avidity for hyperproliferative cells over normal cells. Notwithstanding the foregoing, furocourmarins have the advantage that upon photoactivation with light they may

either form a monoadduct to DNA or crosslink the nuclear DNA, thereby rendering the cell quiescent. Such cytostatic activity does not produce inflammation to the same extent as PDT employing ROPPs and LEPs. A novel class of photosensitizer compounds exhibiting the enhanced specificity of ROPPs and LEPs for hyperproliferating cells and the photocytostatic activity of furocourmarin compounds is described.

The compounds of the present invention form a super-class of compounds characterized by a furocoumarin compound or component thereof, alternatively referred to hereinafter as "F", conjugated with one or more of the following photosensitive molecules: (a) a ROPP (Reactive Oxygen Producing Photosensitizer) or a component thereof; or (b) a LEP (Light Emitting Photosensitizer) or a component thereof to form a F-ROPP or F-LEP. The individual compounds within this super-class of compounds are useful for the diagnosis and treatment of a myriad of diseases as previously described. F-ROPPs contained within this super-class of compounds are classes of compounds containing all possible combinations of any of the compounds set forth in Table 1 conjugated to compounds listed in Table 2. Additional compounds not explicitly listed in Tables 1 and 2 which exhibit the photosensitive and/or tissue specificity properties exemplified by ROPPs or LEPs conjugated to furocoumarins (F-ROPPs) should be construed as included within, and part of, this super-class of compounds. Each class of compound contains a plethora of specific compounds differing only in the particular functional groups attached to the basic structure.

For example, furocoumarins and derivatives thereof can be conjugated with porphyrins, chlorins, bacteriochlorins, isobacteriochlorins, phthalocyanines, naphthalocyanines, porphycenes, porphycyanines, pentaphyrin, sapphyrins,

texaphyrins, phenoxazine dyes, phenothiazines, chaloorganapyrylium dyes, rhodamines, fluorescenes, azoporphyrins, benzochlorins, purpurins, chlorophylls, verdins and triarylmethanes and derivatives thereof, thereby creating 23 new classes of compounds. Compounds within each class are conveniently referred by first specifying the particular furocoumarin followed by the particular ROPP or LEPP. For example, isopsoralen conjugated with chlorin would be isopsorachlorin.

As a further example, furocoumarins such as naturally occurring or synthetic psoralens, as well as derivatives thereof, can be conjugated with one of the following photosensitive compounds from Table 1: porphyrins, chlorins, bacteriochlorins, synthetic isobacteriochlorins, phthalocyanines, naphthalocyanines, porphycenes, porphycyanines, pentaphyrin, sapphyrins, texaphyrins, phenoxazine dyes, phenothiazines, chaloorganapyrylium dyes, rhodamines, fluorescenes, azoporphyrins, benzochlorins, purpurins, chlorophylls, verdins and triarylmethanes, as well as derivatives of such photosensitizers. The foregoing conjugates form new classes of compounds which may conveniently be referred to, for example, as: Psoraporphyrins, Psorachlorins, Psora-bacteriochlorins, Psoraisobacteriochlorins, Psoraphthalocyanines, Psoranaphthalocyanines, Psoraporphycenes, Psoraporphycyanines, Psorapentaphyrin, Psorasapphyrins, Psoratexaphyrins, Psoraphenoxazine dyes, Psoraphenothiazines, Psorachaloorgana-pyrylium dyes, Psorarhodamines, Psorafluorescenes, Psoraazaporphyrins, Psorabenzo-chlorins, Psorapurpurins, Psorachlorophylls, Psoraverdins, and Psoratriarylmethanes, and derivatives thereof, respectively.

The following examples presenting the synthesis of particular photosensitizer compounds in accordance with the present invention are representative of the variety

of photoactive furocourmain-photosensitizer conjugates which can be made and the conditions therefor.

Example 1.

Pyropheophorbide linked 8-MOP. (8-MOP PPhe)

Pyropheophorbide (300mg) was dissolved in dry tetrahydrofuran (100mL) and 1,3-dicyclohexylcarbodiimide (100mg) and dimethylaminopyridine (100mg) were added. After stirring at room temperature for 15 min., a solution of 5-aminomethyl-8-methoxypsoralen (250mg) in dry tetrahydrofuran (60mL) was added. The solution was stirred at room temperature overnight. The solvent was removed by rotary evaporation, and the residual solid dissolved in dichloromethane, washed with dilute HCl then sodium carbonate solution. The organic layer was collected, dried over sodium sulfate, filtered and evaporated to dryness on a rotary evaporator. The crude residue was chromatographed on silica using methanol / dichloromethane (2%) and the major green band collected and evaporated. The residue, 8 Methoxypsorapyropheophoribide (Structure I below), was crystallized from dichloromethane / methanol.

Example 2.

Meso-Pyropheophorbide linked 8-MOP. (8-MOP MPPhe)

Meso-Pyropheophorbide (300mg) was dissolved in dry tetrahydrofuran (100mL) and 1,3-dicyclohexylcarbodiimide (100mg) and dimethylaminopyridine (100mg) were added. After stirring at room temperature for 15 min., a solution of 5-aminomethyl-8-methoxypsoralen (250mg) in dry tetrahydrofuran (60mL) was added.

The solution was stirred at room temperature overnight. The solvent was removed by rotary evaporation, and the residual solid dissolved in dichloromethane, washed with dilute HCl then sodium carbonate solution. The organic layer was collected, dried over sodium sulfate, filtered and evaporated to dryness on a rotary evaporator. The crude residue was chromatographed on silica using methanol / dichloromethane (2%) and the major green band collected and evaporated. The residue, 8-Methoxymesopyropeophoribide (Structure II below), was crystallized from dichloromethane / methanol.

Example 3.

2-(1-Hexyloxyethyl) pyropheophorbide linked 8-MOP. (8-MOP HPPhe)

2-(1-Hexyloxyethyl) pyropheophorbide (200mg) was dissolved in dry tetrahydrofuran (100mL) and 1,3-dicyclohexylcarbodiimide (100mg) and dimethylaminopyridine (100mg) were added. After stirring at room temperature for 15 min., a solution of 5-aminomethyl-8-methoxypsoralen (170mg) in dry tetrahydrofuran (60mL) was added. The solution was stirred at room temperature overnight. The solvent was removed by rotary evaporation, and the residual solid dissolved in dichloromethane, washed with dilute HCl then sodium carbonate solution. The organic layer was collected, dried over sodium sulfate, filtered and evaporated to dryness on a rotary evaporator. The crude residue was chromatographed on silica using methanol / dichloromethane (2%) and the major green band collected and evaporated. The residue, 8-MOP HPPhe (Structure III), was crystallized from dichloromethane / methanol.

Example 4.

Octaethylbenzochlorin linked 8-MOP. (8-MOP OEBCS)

To a stirred solution of octaethylbenzochlorin sulfonylchloride (300mg) in dry dichloromethane (50mL), was added 5-aminomethyl-8-methoxypsoralen (170mg) in dry dichloromethane (20ml) and dry triethylamine (0.1mL). The resulting solution was stirred at room temperature for 1 hr and the solvent removed by rotary evaporation. The crude residue was columned on silica using dichloromethane and the major grey band collected and recrystallized from dichloromethane / methanol to give the title compound (Structure IV below).

Example 5.

Zinc octaethylbenzochlorin linked 8-MOP. (8-MOP ZnOEBCS)

To a stirred solution of octaethylbenzochlorin sulfonylchloride (300mg) in dichloromethane (50mL), was added 5-aminomethyl-8-methoxypsoralen (150mg) in dichloromethane (20ml) and dry triethylamine (0.1mL). The resulting solution was stirred at room temperature for 1 hr. Zinc acetate (200mg) dissolved in methanol (lOmL) was added to the reaction solution and the solution was warmed on a hot water bath until metallation of the benzochlorin was complete by Uv / vis spectroscopy (as seen by a band I absorption at 673nm). The solvent was then removed by rotary evaporation and the crude residue redissolved in dichloromethane (5mL) and chromatographed on silica using dichloromethane. The major green band collected and recrystallized from dichloromethane / methanol to give the title compound (Structure V below).

Example 6.

Cu iminium octaethylbenzochlorin linked 8-MOP. (8-MOP Cu Im OEBCS)

To copper octaethylbenzochlorin sulfonic acid (300mg) dissolved in dichloromethane (100mL) was added (chloromethylene) dimethylammonium chloride (500mg) and the solution stirred overnight at room temperature, protected from moisture. The solution was poured into ice cold water quickly, the organic layer washed with water rapidly, separated and dried over sodium sulfate. The solution was filtered to remove sodium sulfate and 5-aminomethyl-8-methoxypsoralen (200mg) in dichloromethane (20mL) was added. The solution was stirred for 20 minutes at room temperature, then poured into water. The organic layer was washed with dilute HCl and dried over sodium sulfate. The solution was filtered and evaporated to dryness. The resulting reside was chromatographed on silica using 2% methanol / dichloromethane and the major green band collected and evaporated. The title compound (Structure VI below) was obtained as a green powder by precipitation from dichloromethane / hexane.

Example 7.

Indium texaphyrin linked 8-MOP. (8-MOP InT)

To a solution of Indium texaphyrin-16-carboxylic acid (200mg) was dissolved in dry terahydrofuran (50mL) and 1,3-dicyclohexylcarbodiimide (50mg) and dimethylaminopyridine (50mg) added. After stirring at room temperature for 15 min., a solution of 5-aminomethyl-8-methoxypsoralen (100mg) in dry terahydrofuran (20mL) was added. The solution was stirred under argon at room temperature overnight. The solvent was removed by rotary evaporation, and the residual solid dissolved in

dichloromethane and washed with dilute HCl and finally with water. The organic phase was separated, dried over sodium sulfate, revaporated under reduced pressure and chromatographed on silica using methanol / dichloromethane (2%). The major green band was collected and evaporated. The residue, 8-MOP InT (Structure VIII below), was crystallized from dichloromethane / hexane.

Example 8.

Protoporphyrin linked 8-MOP. (8-MOP PP)

Protoporphyrin (200mg) was dissolved in oxalyl chloride (3mL) and the solution warmed at 40°C for lhr, while being protected from moisture. The excess oxalyl chloride was removed under high vacuum and dry dichloromethane (5mL) was added. This was also removed under high vacuum, to give a purple residue that was protected from moisture via a drying tube. Dry dichloromethane (10mL) and dry triethylamine (1mL) were added to the residue, followed by a solution of 5-aminomethyl-8-methoxypsoralen (160mg) in dry dichloromethane (20mL). The solution was stirred overnight, protected from moisture via a drying tube. The solution was then poured into water and the organic phase washed well with water, collected and dried over sodium sulfate. After filtration and evaporation to dryness, the resulting residue was columned on silica using 2% acetone / dichloromethane as eluent. The major red band was collected and recrystallized from dichloromethane / methanol to yield the title compound VIII.

Example 9.

Tetraphenylporphyrin linked 8-MOP. (8-MOP TPP)

Meso-terakis-(4'-carboxyphenyl) porphyrin (200mg) was dissolved in oxalyl chloride (5mL) and the solution warmed at 40°C for lhr, while being protected from moisture. The excess oxalyl chloride was removed under high vacuum and dry dichloromethane (5mL) was added. This was also removed under high vacuum, to give a green residue that was protected from moisture via a drying tube. Dry dichloromethane (10mL) and dry triethylamine (lmL) were added to the residue and a solution of 5-aminomethyl-8-methoxypsoralen (400mg) in dry dichloromethane (20mL) was added. The solution was stirred overnight, protected from moisture via a drying tube. The solution was then poured into water and the organic phase washed well with water, collected and dried over sodium sulfate. After filtration and evaporation to dryness, the resulting residue was columned on silica using 2% acetone / dichloromethane as eluent. The major red band comprised 8-MOP TPP (Structure IX) and was collected and recrystallized from dichloromethane / methanol.

Example 10.

2,8,12,18-Tetraethyl-3,7,13,17-tetramethyl-5,15-bis(2'-furan) porphyrin. (5,15-DFP).

4,4'-Diethyl-3,3'-dimethyl-2,2'-dipyrrylmethane (4.0g) and 2-furaldehyde (1.67g) were dissolved in methanol (100mL) and the solution deaerated by bubbling with argon for 15min. 4-Toluenesulfonic acid (0.95g) was added and the solution stirred for 2hrs in the dark, then refrigerated overnight. The precipitated porphyrinogen was collected, washed with ice cold methanol (20mL) and resuspended in methanol

(100mL). o-Chloranil (6.0g) was added and the solution stirred in the dark for 2hrs. Triethylamine (2mL) was added and the precipitated porphyrin was collected by filtration, washed well with methanol and dried under high vacuum. The porphyrin was recrystallized from dichloromethane / methanol to yield the title compound (X).

Example 11.

Texas red linked 8-MOP. (8-MOP TR)

Sulforhodamine 101 acid chloride (200mg) was dissolved in dry tetrahydrofuran (100mL) and 5-aminomethyl-8-methoxypsoralen (100mg) added, followed by triethylamine (0.1mL). The solution was left overnight at room temperature. The following day the solution was evaporated to dryness, redissolved in dichloromethane and columned on silica using 2% methanol / dichloromethane as eluent. The major fluorescent red fraction was collected and evaporated to dryness. The residue, comprising 8-MOP TR (Structure XI) was recrystallized from dichloromethane / hexane.

Example 12.

Rhodamine B linked 8-MOP. (8-MOP RB)

Sulforhodamine B acid chloride (200mg) was dissolved in dry tetrahydrofuran (100mL) and 5-aminomethyl-8-methoxypsoralen (100mg) added, followed by dry triethylamine (0.1mL). The solution was left overnight at room temperature. The following day the solution was evaporated to dryness, redissolved in dichloromethane and columned on silica using 2% methanol / dichloromethane as eluent. The major

fluorescent red fraction was collected and evaporated to dryness. The residue (Structure XII) was recrystallized from dichloromethane / hexane.

Example 13.

Porphocyanine linked 8-MOP. (8-MOP Pocy)

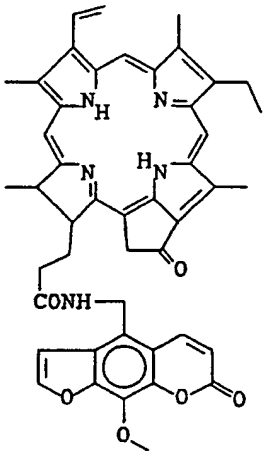
2,3,21,22-tetraethyl-12-(4'-carboxyphenyl) porphocyanine (200mg) was dissolved in dry tetrahydrofuran (100mL) and 1,3-dicyclohexylcarbodiimide (100mg) and dimethylaminopyridine (100mg) were added. After stirring at room temperature for 15 min., a solution of 5-aminomethyl-8-methoxypsoralen (300mg) in dry tetrahydrofuran (60mL) was added. The solution was stirred at room temperature overnight. The solvent was removed by rotary evaporation, and the residual solid dissolved in dichloromethane, washed with dilute HCl then sodium carbonate solution. The organic layer was collected, dried over sodium sulfate, filtered and evaporated to dryness on a rotary evaporator. The crude residue was chromatographed on silica using methanol / dichloromethane (2%) and the major green band collected and evaporated. The residue (Structure XIII) was crystallized from dichloromethane / methanol.

Example 14.

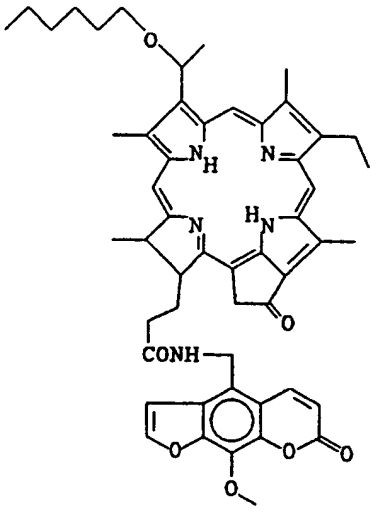
Phthalocyanine linked 8-MOP. (8-MOP Pth)

Phthalocyanine tetra sulfonate (200mg) was dissolved in phosphorus oxychloride (20mL) and the solution refluxed for 2 hrs. The excess phosphorus oxychloride was removed by rotary evaporation and the residue dissolved in dry, cold pyridine (10mL). A solution of 5-aminomethyl-8-methoxypsoralen (300mg) in dry

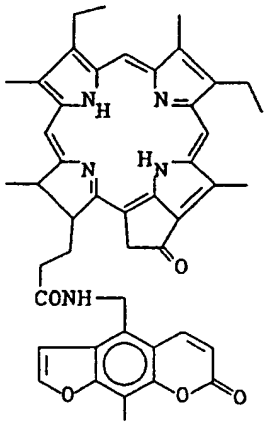
pyridine (60mL) was added. The solution was stirred at room temperature overnight. The solvent was removed by rotary evaporation, and the residual solid dissolved in dichloromethane, washed with dilute HCl then sodium carbonate solution. The organic layer was collected, dried over sodium sulfate, filtered and evaporated to dryness on a rotary evaporator. The crude residue was chromatographed on silica using methanol / dichloromethane (5%) and the major green band collected and evaporated. The residue (Structure XIV) was crystallized from dichloromethane / methanol.



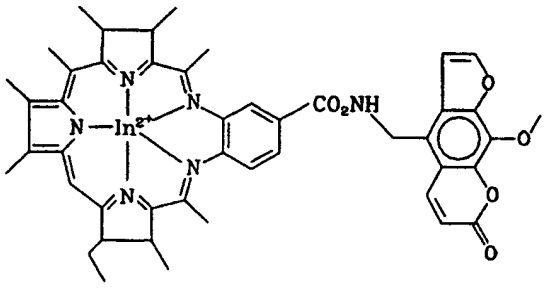
(8-MOP PPhe)
I



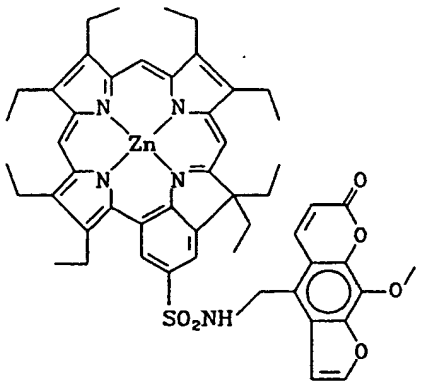
8-MOP HPPhe
III



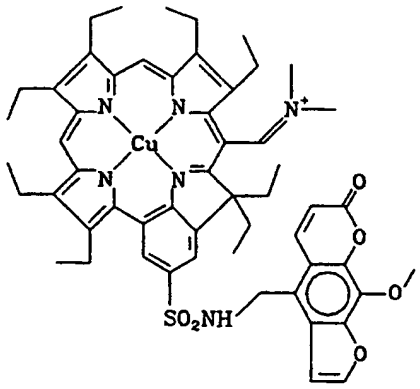
8-MOP PPhe
II



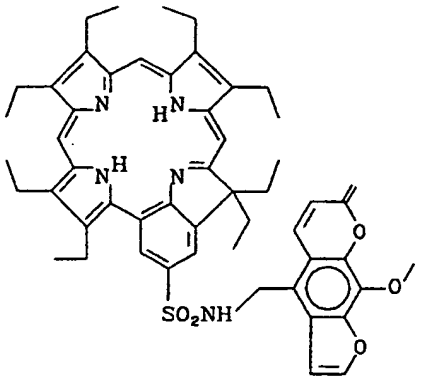
8-MOP InT
VII



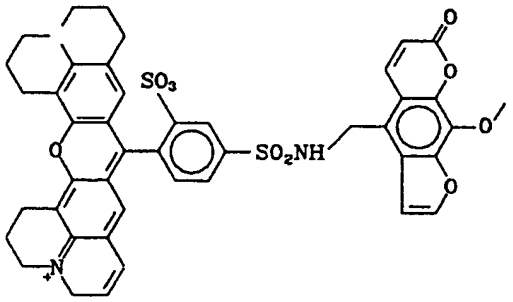
8-MOP ZnOEBCS
V



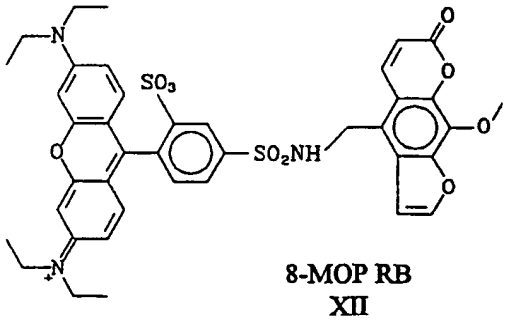
8-MOP Cu Im OEBCS
VI



8-MOP OEBCS
IV

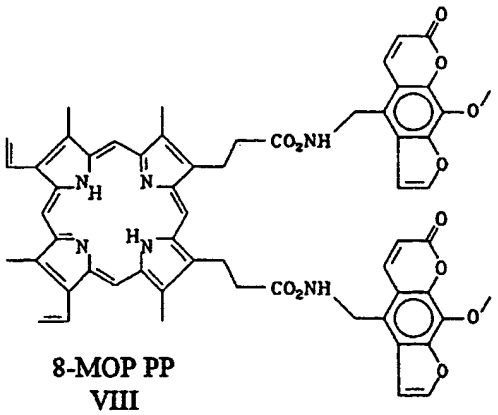


8-MOP TR
XI

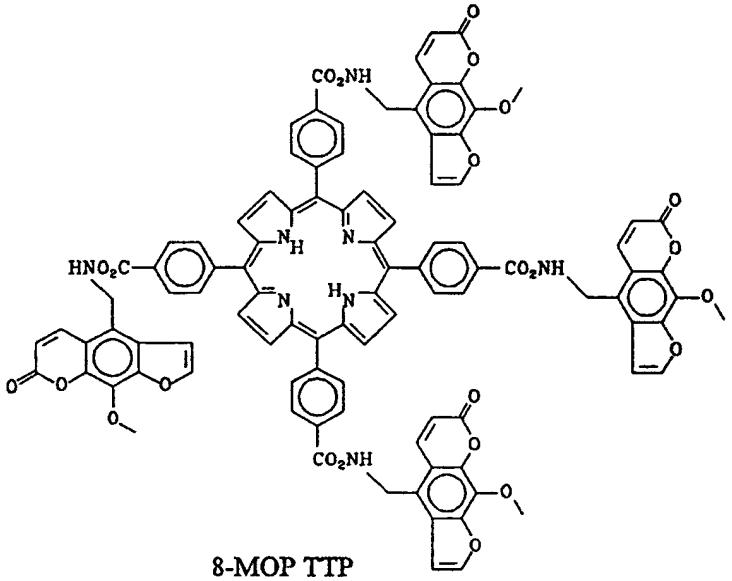


8-MOP RB
XII

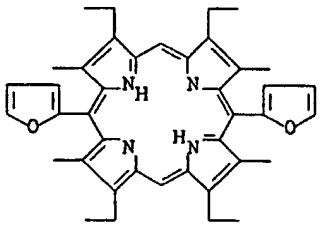
1
2



8-MOP PP
VIII

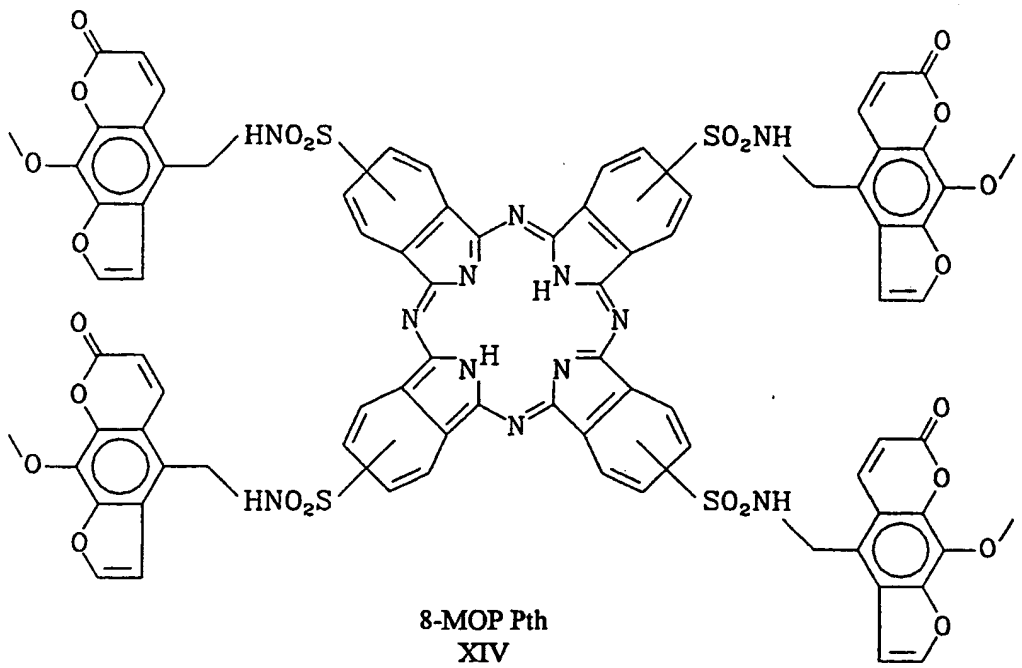


8-MOP TTP
IX

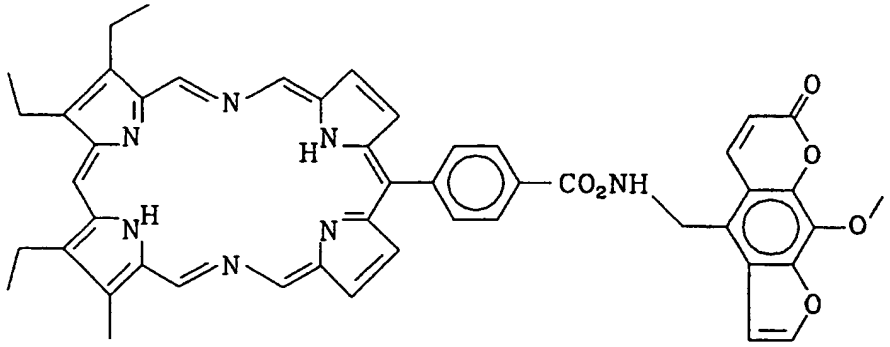


5,15-DFP
X

1



8-MOP Pth
XIV



8-MOP Pocy
XIII

The preceding super-class of photosensitizing compounds may be characterized by: a) a furocoumarin attached to a Reactive Oxygen Producing Photosensitizer type compound, F-ROPP; b) a furocoumarin sub-component attached to a ROPP, FS-ROPP; c) a cationic furocoumarin attached to an ROPP (neutral or cationic), to produce either CF-ROPP or CFS-ROPP; d) a cationic ROPP attached to a furocoumarin (neutral or cationic); e) any one of the above compounds wherein the ROPP is metalized; and f) a furocoumarin conjugated with a light emitting photosensitizer, F-LEP.

The foregoing super-class of conjugated compounds can be used to treat a variety of diseases such as atherosclerosis, restenosis, cancer, cancer precursors, non-cancerous hyperproliferative diseases, psoriasis, macular degeneration, glaucoma, and certain viruses. These compounds are light activatable drugs which may or may not be photodynamically active (i.e. produce singlet oxygen and/or oxygen radicals to mediate cytotoxicity), but will be photoactive (i.e. exhibit photochemical cross-linking with DNA or RNA or the production of monoadducts of the compound therewith) to modulate the metabolic activity of cells. More specifically, these novel photoactive compounds will retain the ability of the ROPP or LEP to localize to a greater extent in the target tissue and the ability of the furocoumarin (such as psoralen) to intercalate into target tissue DNA and form cross-linked and/or monoadducts adducts upon the addition of light energy.

Previous studies indicate that utilizing a cationic ROPP or LEP to synthesize a CF-ROPP or CF-LEP facilitates the intercalation of the compound into target cell DNA. Once the F-ROPP or CF-ROPP is localized in target cells, light activation can be used therapeutically and/or diagnostically. The use of these novel compounds for

the detection and/or treatment and the prevention of restenosis and intimal hyperplasia following cardiac transplantation surgery (or AV shunt procedures such as dialysis) is an exemplary application which is discussed in particular detail to teach and illustrate a use for the invention, but it should be kept in mind that such an application is illustrative and should not be construed as a limitation of this invention.

For example, another application for the photosensitizer compounds described herein is the light activated treatment of a target tissue which does not selectively concentrate either ROPPs or furocoumarins. An F-ROPP, selected as described below from the super-class of compounds described above, can be administered systemically to a biological organism, which organism could be an animal, a plant or even a single cell or a polynucleic acid fragment. Following systemic administration of the F-ROPP, and while the F-ROPP is present in the animal's serum, a light source operating at a strong absorption wavelength of the furocoumarin component of the F-ROPP, is directed toward the volume of target tissue in which high concentrations of the F-ROPP are desired. The selection of the particular furocoumarin used in the F-ROPP is preferably a species which creates mono-adducts with polynucleic acids when activated with UV or short wavelength visible light. By administering the activating light to the target tissue, mono-adducts of F-ROPPs with DNA and RNA are formed. Increasing the intensity of the activating light delivered to the target tissue increases the DNA-bound F-ROPP therein. When the F-ROPP reaches the desired concentration in the target tissue, a longer wavelength of light which activates the ROPP portion of the F-ROPP may be used to photoactivate the cell bound F-ROPP in the target tissues to selectively destroy or modify the target tissue. In effect, the F-ROPP creates a light-induced selectivity of the F-ROPP for binding to the target tissue because only the

target tissue is illuminated with the shorter wavelength of light thereby causing covalent bonding of F-ROPP only in the DNA/RNA of the target tissue.

While particular embodiments of the present invention have been illustrated and described, it would be obvious to those skilled in the art that various other changes and modifications can be made without departing from the spirit and scope of the invention. It is therefore intended to cover in the impending claims all such changes and modifications that are within the scope of this invention.

CLAIMS

1. Photoactive compounds comprising a functional furocoumarin conjugated with a photosensitive benzochlorin compound.

2. The photoactive compounds of Claim 1 wherein the photosensitive benzochlorin compound is cationic.

3. The photoactive compound of Claim 1 wherein said photosensitive compound comprises a metal coordinated to a benzochlorin molecule.

4. The photoactive compound of Claim 3 wherein the metal is selected from the group consisting of copper, aluminum, tin, zinc, gadolinium, manganese, magnesium or iron.

5. The photoactive compounds of Claim 1 wherein said functional furocoumarin comprises a psoralen.

6. A photoactive compound having the structure $R\text{-}R^1$ wherein R comprises a furocoumarin and wherein R^1 is a reactive oxygen-producing benzochlorin compound.

7. A photoactive compound in accordance Claim 9 wherein R^1 is a light-emitting benzochlorin compound.

8. A photoactive compound in accordance Claim 9 wherein said furocoumarin is selected from the group consisting of compounds comprising isopsoralen, pseudopsoralen, pseudoisopsoralen, allopsoralen and pseudoallopsoralen; or derivatives thereof.

9. A photoactive composition for treating diseased target tissue cells within an organism, said photoactive composition having the form R-R' wherein R is a photoactivatable furocoumarin compound which covalently bonds to target tissue cells only when the furocoumarin compound is photoactivated with light having a first

wavelength, and R' is a photosensitive benzochlorin compound which interferes with normal cellular activity within the diseased target tissue only when photoactivated with light having a second wavelength, which second wavelength is different from said first wavelength.

10. Photoactive compounds comprising a functional furocoumarin conjugated with a photosensitive pyrrole-derived macrocyclic compound.

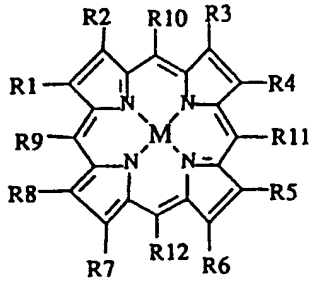


FIG.1

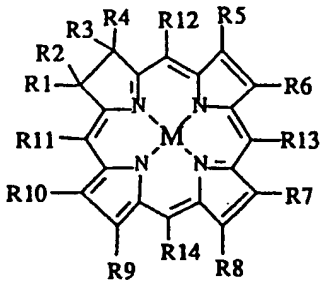


FIG.2

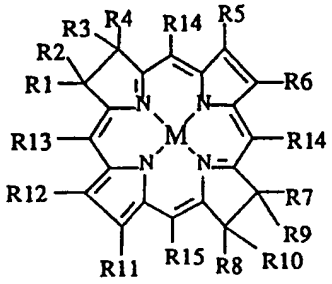


FIG.3

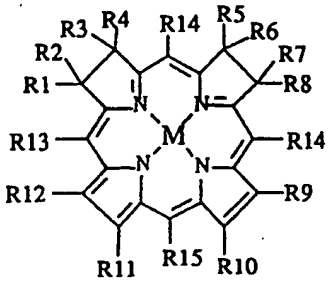


FIG.4

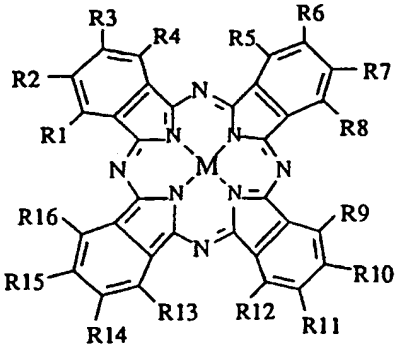


FIG.5

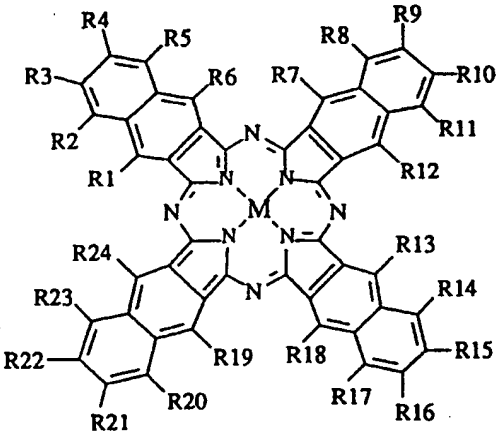


FIG.6

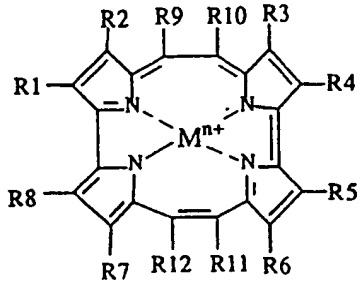


FIG.7

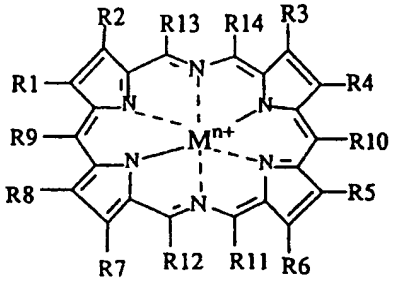


FIG.8

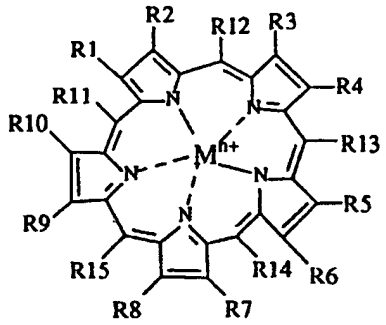


FIG.9

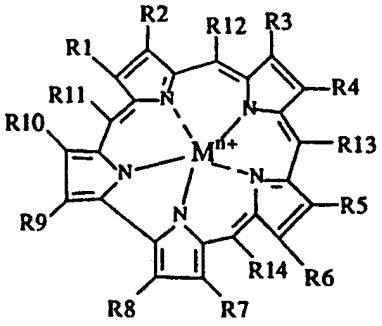


FIG.10

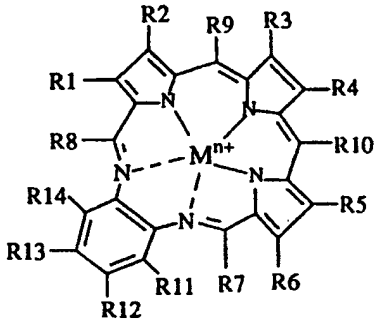


FIG.11

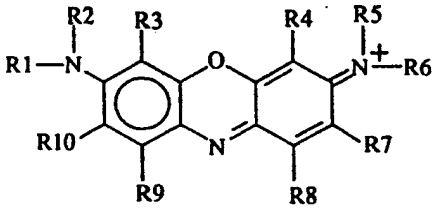


FIG.12

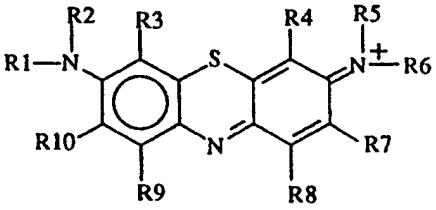


FIG.13

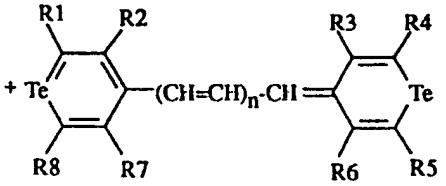


FIG.14

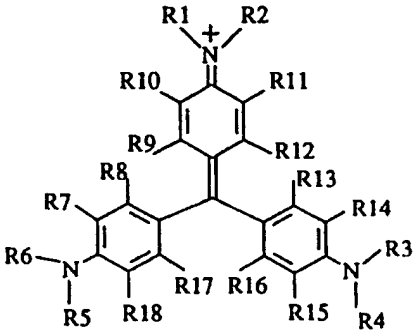


FIG.15

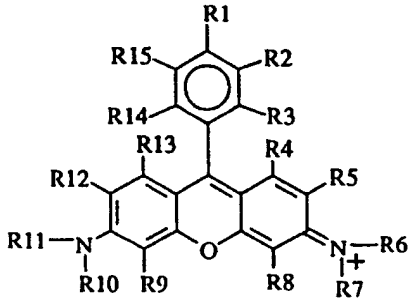


FIG.16

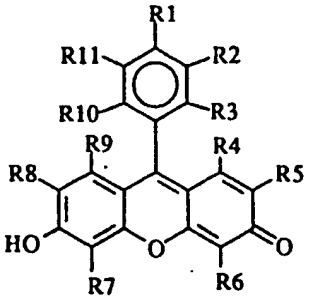


FIG.17

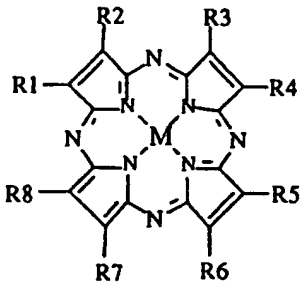


FIG.18

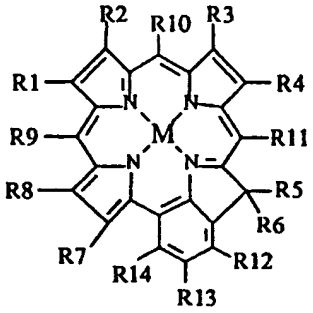


FIG.19

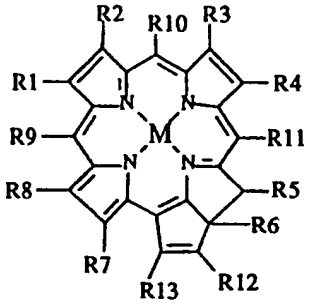


FIG.20

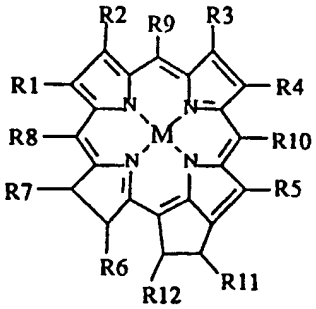


FIG.21

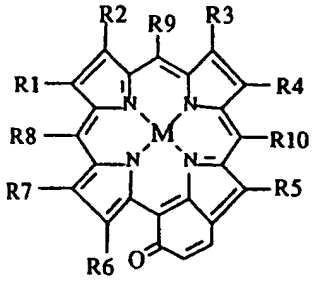


FIG.22

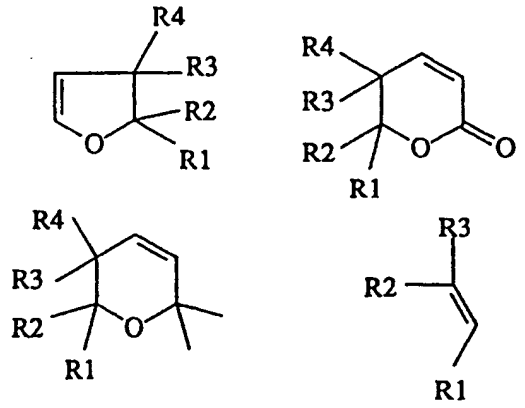


FIG.23

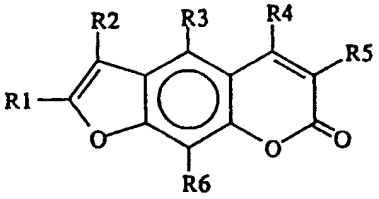


FIG.24

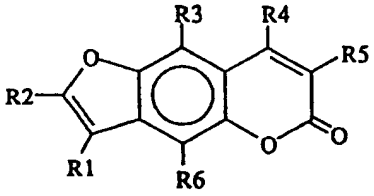


FIG.25

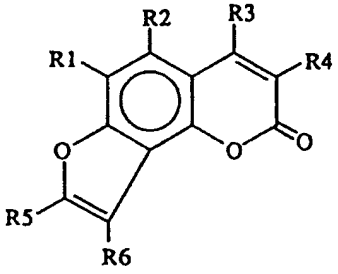


FIG.26

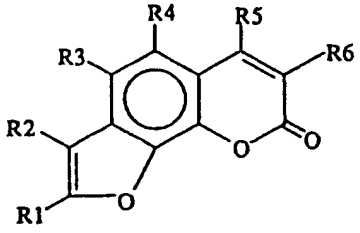


FIG.27

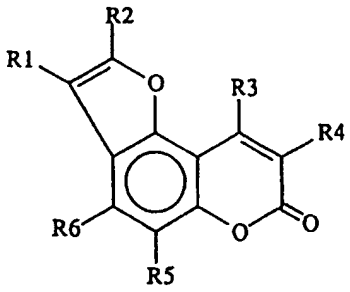


FIG.28

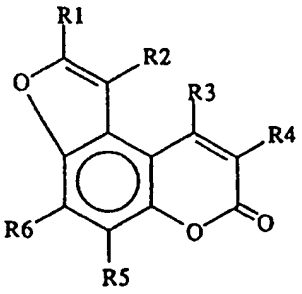


FIG.29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12128

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07D 309/38; C07D 487/22
US CL :549/387; 540/ 145

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 549/387; 540/145

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A 5,356,929 (HEINDEL et al) 18 October 1994, See columns 1 and 2.	1-10
Y	US, A, 5,286,474 (GUST et al.) 15 February 1994, See entire document.	1-10
A, E	US, A, 5,539,100 (WASIELEWSKI et al.) 23 July 1996, See entire document.	1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 OCTOBER 1996	06 NOV 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer P. K. SRIPADA tcj Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*